Bone Densitometry as a Diagnostic Tool for Monitoring Osteoporosis in Live layer Breeders

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Two-Year Technical Report
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Background:

Osteoporosis, associated with egg laying strains of chickens, is caused by mineral loss from bones. The bone loss process in laying hens is induced by a high demand for calcium during eggshell formation. The problem is now being compounded by increased welfare awareness and the difficulty to dispose spent hens at the end of lay. The welfare issues relate to increased fractures during production, handling and transportation to processing facilities causing pain to the birds. From a food safety point of view, some companies that process spent hens for meat are now reluctant to use them because of bone splintering which renders the meat product unsafe.

Project Objective:

A. Long-term goal: To determine the applicability of bone densitometry as a tool to genetically select live egg-laying strains of birds for bone integrity.

B. Supporting objectives of proposed project:

(1) To determine if densitometry readings are dependent on the stage of egg calcification in sexually mature birds.

(2) To determine the degree of variation in bone mineral content and density among birds at different ages of egg-laying life cycle.

(3) To determine when osteoporosis is most critical during the egg production cycle and whether bone loss is associated with blood levels of hydroxyproline and shell quality traits

What We've Done

Questions: Of the 24 hours needed for a White Leghorn hen to fabricate an egg, it spends 20 hours in the uterus being plumped and forming the egg shell. The source of calcium for the shell is mainly dietary through intestinal absorption, but medullary bone can serve as a source of calcium as well. Does a hen which is depositing calcium carbonate onto the shell of her egg have lower medullary bone mineral during the time that egg calcification is occurring as compared to other stages of the egg formation such as after an egg is laid? This question of whether or not the mineral density and content of medullary bone varies depending on the stage of egg development needs to be answered. If we find that bone mineral density and content does vary with stage of eggshell calcification, then it is important for us in later experiments to conduct bone scans with the densitometer at the most appropriate time. Thus, results from this experiment will dictate when the bone scans need to be done. Scans may need to be done immediately post-oviposition or when the eggshell is being calcified in the uterus (determined by palpation). Or results may indicate that medullary bone mineral content does not vary with stage of calcification and can be done at any time of the day.

Methodology: Densitometric bone scans of the tibia (medullary bone) and humerus (pneumatic bone) from 8 live control-fed birds undergoing active egg formation were performed at 0, 5, 15, and 20 hours post-oviposition at 24, 30 and 40 weeks of age. The first bone scan at 0 hour was performed immediately following oviposition. The second scan at 5 hours post oviposition reflected bone mineral content immediately after the egg entered the uterus. The third scan at 15 hours post oviposition was performed during active calcification and the final scan at 20 h post oviposition was done near the end of the calcification period.

Results: Bone mineral density (Table 1) and content (data not shown) of the tibia and humerus of White Leghorn hens did not change as an egg was being formed in the reproductive tract of the hen (Table 1). These results were apparent at 24, 30, and 40 wk of age indicating that densitometry readings are not dependent on the 24-hour ovulatory cycle. Therefore, scans do not have to be conducted when the eggshell is being formed and can be done at any time of the day, which will make commercial application easier. As expected, the medullary tibia had significantly greater bone mineral density than the hollow humerus at all ages ($P < 0.0009$, Table 1).

TABLE 1. Bone mineral density of the tibia and humerus of female White Leghorn primary breeding stock during an ovulatory cycle as determined through scans of live birds using x-ray densitometry

	Time post-oviposition				
Age of hen	0 h	5 h	15 h	20 h	$\bar{x}$
	(g/cm^2)				
24 wk of age					
Tibia	0.158[1]	0.158	0.156	0.151	0.156[a]
Humerus	0.138	0.137	0.143	0.134	0.138[b]
SEM	0.007	0.007	0.007	0.007	0.004
30 wk of age					
Tibia	0.169	0.165	0.169	0.165	0.167[a]
Humerus	0.133	0.135	0.134	0.135	0.134[b]
SEM	0.006	0.006	0.006	0.006	0.003
40 wk of age					
Tibia	0.163	0.159	0.181	0.165	0.167 [a]
Humerus	0.126	0.132	0.124	0.123	0.126 [b]
SEM	0.006	0.006	0.006	0.006	0.003

[1] Values represent the mean of 8 observations.
[a,b] Means in a column within age are significantly different at a $P < 0.0009$.

Question: Will densitometric scans of bones from live birds correlate with the densitometric scans of excised bones?

Methodology: Measurement of bone mineral density and content of the tibia and humerus of 5 birds were conducted between 17 and 67 weeks of age at 10-week intervals using the Norland bone densitometer. The birds were euthanized and their humerus and tibia excised and re-scanned.

Results: The bone mineral density between live scans and excised bones were similar (see bottom three rows of Table 2, page 6 and Figure 1 on page 7), thus validating the accuracy of the Norland densitometer. Correlations of scans of bones in live birds with excised bones averaged 0.75 ($P < 0.0001$) for the tibia and 0.91 ($P < 0.0001$) for the humerus. Again, the medullary tibia had significantly greater bone mineral density and content than the hollow humerus.

TABLE 2. Bone traits from scans of live birds as compared to excised bones of female White Leghorn primary breeding stock between 17 and 67 wk of age as determined through x-ray densitometry

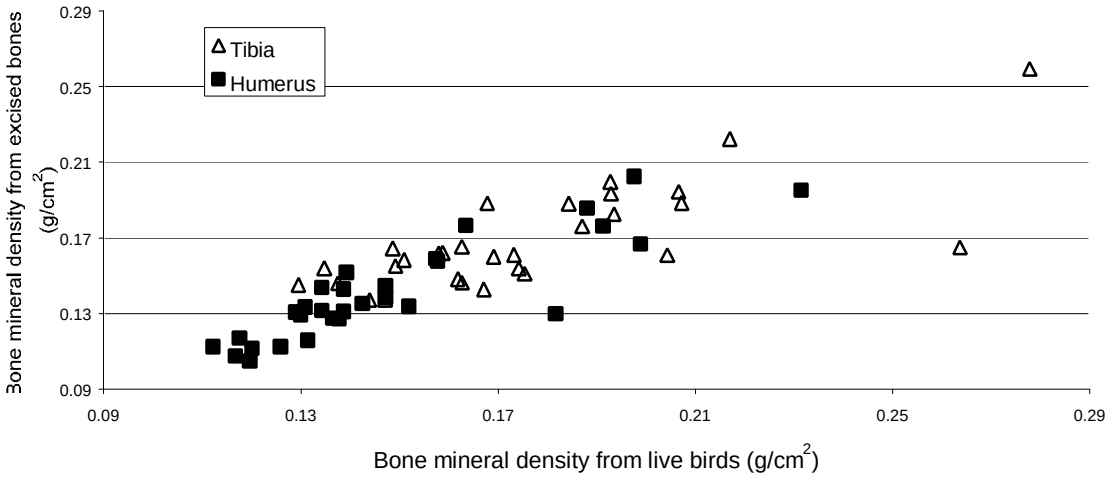
Age Bone Status	Bone mineral density (BMD) (g/cm^2)	Bone mineral content (BMC) (g)	Area (cm^2)
17 wk of age[1]			
Tibia			
Live	0.154	1.98	12.9
Excised	0.161	2.08	12.9
Humerus			
Live	0.133	1.14	8.6
Excised	0.130	1.11	8.6
27 wk of age[1]			
Tibia			
Live	0.153	2.06	13.4
Excised	0.162	2.18	13.4
Humerus			
Live	0.156	1.30	8.4
Excised	0.149	1.29	8.8
37 wk of age[1]			
Tibia			
Live	0.167	2.18	13.0
Excised	0.155	2.00	12.9
Humerus			
Live	0.130	1.06	8.1
Excised	0.122	1.06	8.7
47 wk of age[1]			
Tibia			
Live	0.182	2.45	13.5
Excised	0.166	2.20	13.3
Humerus			
Live	0.139	1.25	9.0
Excised	0.146	1.22	8.4

(**TABLE 2** continued)

Age Bone Status	Bone mineral density (BMD) (g/cm^2)	Bone mineral content (BMC) (g)	Area (cm^2)
57 wk of age[1]			
Tibia			
Live	0.187	2.48	13.2
Excised	0.157	2.02	12.8
Humerus			
Live	0.134	1.18	8.8
Excised	0.127	1.15	9.1
67 wk of age[1]			
Tibia			
Live	0.218	2.98	13.7
Excised	0.213	2.78	13.1
Humerus			
Live	0.198	1.83	9.3
Excised	0.188	1.52	7.8
Bone[2]			
$\bar{x}$ Tibia	0.173^{a}	2.28^{a}	13.2^{a}
$\bar{x}$ Humerus	0.146^{b}	1.26^{b}	8.6^{b}
Scan[2]			
$\bar{x}$ Live	0.163	1.72^{b}	11.0
$\bar{x}$ Excised	0.157	1.82^{a}	10.8
SEM[3]	0.003	0.04	0.08

[1]Values represent the mean of 5 observations.
[2]Values represent the mean of 60 observations
[3]Standard error of the mean (SEM) for the main effect of type of scan (live bird *vs.* excised bone).
[a,b] Means in a column within type of bone or scan with different superscripts are significantly different at a $P < 0.04$.

FIGURE 1. Bone mineral density was measured in live chickens and then in excised bones from the same birds. Correlations between the live and excised measurements were 0.75 (P < 0.0001) for the tibia and 0.91 (P < 0.0001) for the humerus.



Questions: Does significant variation in bone mineral density and content exists among individual egg laying strains of chickens as measured through densitometric scans of live birds? Exactly when in the life cycle of the chicken does bone loss start? When does bone loss become critical leading to osteoporosis, and what would be the best age for genetic selection for increased bone mass? If considerable variation in bone mineral density exists among individual birds, genetic selection might be enacted to select hens for increased bone mass, which is a long-term goal of this project. Establishing the bone integrity profile over the production cycle may provide a better insight on how best to manage the incidence of osteoporosis and improve the overall well being of laying hens.

Methodology: Measurement of bone mineral density and content of the tibia and humerus from 31 to 35 live birds fed a control diet was conducted from 15 to 65 weeks of age at 10-week intervals.

Results: We have monitored the bone mineral density (BMD) and content (BMC) of a pedigree stock of White Leghorns through 65 wk of age (Table 3). The large increase in BMD and BMC of hens at 25 wk as compared to 15 wk of age was most likely due to the change in diet at 18 wk of age. Because hens are sexually maturing at this age and are starting to lay eggs, they are placed on a high calcium layer breeder diet as compared to the previously fed grower and developer pullet diets. Post- peak declines in BMD and BMC of the humerus and tibia did not occur by 65 wk of age. Again, the hollow humerus had less bone mineral density and content than the medullary tibia (Table 3). Coefficients of variation for BMD and BMC of egg-type hens after 25 wk of age were greater than 10% (Table 3). This variability among hens in BMD and BMC suggests that there is genetic potential to improve skeletal integrity of birds through the use of densitometry.

TABLE 3. Bone traits of the humerus and tibia of female White Leghorn primary breeding stock from 15-to 65- wk of age as determined through scans of live birds using x-ray densitometry

Age of hen (wk)	Bone mineral density[1] (BMD) (g/cm^2)	Coefficient of variation for BMD (%)	Bone mineral content[1] (BMC) (g)	Coefficient of variation for BMC (%)
Humerus				
15	0.110 [d]	7.5	0.93 [d]	9.7
25	0.140 [c]	15.4	1.15 [c]	14.4
35	0.137 [c]	11.2	1.17 [c]	11.8
45	0.147 [b]	13.2	1.22 [bc]	11.3
55	0.153 [b]	13.5	1.29 [b]	14.1
65	0.169[a]	15.4	1.63[a]	18.0
SEM	0.004		0.03	
Tibia				
15	0.132[d]	7.5	1.67 [c]	9.5
25	0.155 [c]	10.2	2.03 [b]	12.7
35	0.161 [c]	12.7	2.15 [b]	17.1
45	0.174 [b]	12.7	2.32 [a]	16.6
55	0.186 [a]	14.5	2.47 [a]	18.3
65	0.187[a]	17.6	2.47 [a]	22.5
SEM	0.004		0.07	

[1] Values represent the mean of 31 to 35 observations.

[a-d] Means in a column within the humerus or tibia are significantly different at a $P < 0.05$. The interaction of bone with age was significant ($P < 0.005$).

Because of the start-up funding received from the "Indiana Value-Added Grant Fund," we were successful in obtaining federal dollars from the USDA National Research Initiative to continue this research into the second cycle of egg production. After collecting the data on BMD and BMC at 65 wk of age, the hens were subjected to an induced molt and their skeletal integrity was evaluated not only during and immediately following a molt, but is also currently being studied during the second cycle of egg production. The following figure graphs the BMD shown in Table 3 and includes the effect of an induced molt, which occurred between 76 and 80 wk of age. Note the dramatic decrease in BMD during the time of the molt. By 85 wk of age, the hens were in their second cycle of egg production and their BMD had still not fully recovered from pre-molt values at 75 wk of age.

We have calculated correlations among BMD measurements at different ages to determine if early measurements are accurate predictors of later measurements (Table 4). Weak correlations were found between BMD measured at 15 wk and later ages, but correlations between 25 wk and later ages were improved, particularly for the humerus ($P < 0.001$, $r > 0.60$). Thus, early measurement of BMD at 25 wk of age may be useful predictors of BMD later in life.

TABLE 4. Correlation values for bone mineral density of the humerus and tibia of female White Leghorn primary breeding stock at 15 to 65 wk of age as determined through scans of live birds using x-ray densitometry

	Correlation values				
	25 wk	35 wk	45 wk	55 wk	65 wk
Humerus					
15 wk	0.44**	0.37*	0.28	0.51**	0.45**
25 wk		0.78***	0.64***	0.75***	0.60***
35 wk			0.67***	0.85***	0.66***
45 wk				0.83***	0.75***
55 wk					0.80***
Tibia					
15 wk	0.22	0.47**	0.33	0.42*	0.24
25 wk		0.48**	0.47**	0.70***	0.56***
35 wk			0.44**	0.37*	0.32
45 wk				0.54***	0.55***
55 wk					0.76***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Calcium Deficient Diet

Question: Can densitometry accurately measure differences in bone mineral density and content in live birds fed varying levels of dietary calcium?

Methodology: Two additional groups of hens were fed a calcium deficient diet of 1.85 % Ca and a hypercalcemic diet of 5.40 % Ca beginning at 32 wk of age. Repeated densitometric readings were made on the tibia and humerus at 35, 45, and 55 wk of age. Bone density results from birds fed hypocalcemic and hypercalcemic diets were compared with control birds fed a diet with adequate calcium (3.65% Ca after 18 weeks of age).

Results: The BMD and BMC of hens consuming hypercalcemic (5.4% Ca), control (3.65% Ca), or hypocalcemic (1.85% Ca) diets increased as hens consumed more calcium (Table 5).

TABLE 5. The effect of dietary calcium fed from 32 to 56 wk of age on bone mineral density and content of female White Leghorn primary breeding stock as determined through scans of live birds using x-ray densitometry

Bone Dietary treatment	n	Bone mineral density (BMD) (g/cm^2)	Bone mineral content (BMC) (g)
Tibia			
Hypercalcemic	30	0.196[a]	2.60[a]
Control	29	0.176[b]	2.36[b]
Hypocalcemic	25	0.162[c]	2.12[c]
SEM		0.005	0.08
Humerus			
Hypercalcemic	30	0.160[a]	1.31[a]
Control	28	0.140[b]	1.20[a]
Hypocalcemic	25	0.142[b]	1.21[a]
SEM		0.005	0.04

[a-c] Means among diets within a bone within a column are significantly different at a $P < 0.05$. The diet x bone interaction was significant at a $P < 0.01$.

Likewise, shell quality traits (% shell and shell thickness) were positively correlated with BMD and BMC (r= 0.42, P <0.0009, Table 6).

TABLE 6. The effect of dietary calcium fed for 4 wk on bone and shell traits of female White Leghorn primary breeding stock

	Bone mineral density[1] (BMD) (g/cm^2)	Bone mineral content[1] (BMC) (g)	Shell[2] (%)	Shell thickness[2] (mm)
Hypercalcemic	0.172[a]	1.90[a]	9.3[a]	0.362[a]
Control	0.161[a]	1.82[a]	9.5[a]	0.371[a]
Hypocalcemic	0.137[b]	1.53[b]	8.0[b]	0.305[b]
SEM	0.005	0.06	0.2	0.008

[1]Values represent the mean averaged across two bones (tibia vs. humerus) x 10 birds/bone or n = 20 observations. The interaction of diet with bone was non-significant.
[2]Values represent the mean of eggs laid by 10 hens (1 to 2 eggs/hen) or n = 10 observations.
[a,b] Means within a column with different superscripts are significantly different at a $P < 0.05$.

The data presented in this report have been statistically analyzed using an analysis of variance. Because body weight has been correlated with osteoporosis in humans, we are currently reanalyzing the data using an analysis of covariance adjusting for the covariant of body weight.

Together, these data indicate that we can accurately measure BMD and BMC in live chickens by densitometry. Our long-term goal is to reduce the incidence of osteoporosis in egg-type chickens through genetic selection. Thus, we have initiated a long-term project to identify quantitative trait loci (QTL) associated with BMD for potential use in marker-assisted selection program. A federal grant submitted to the National Research Initiative titled “Identification of Quantitative Trait Loci Influencing Bone Mineral Density in Chickens” is currently pending.

Published Abstracts:

Schreiweis, M. A., J. I. Orban, M. C. Ledur, and P. Y. Hester, 2001. Assessment of densitometry to measure bone mineral content and density in live birds as a tool for monitoring osteoporosis in laying hens. Poultry Sci. 80: (suppl. 1): 94.

Orban, J. I., and P. Y. Hester, 2001. Profile of plasma hydroxyproline in laying hens during an ovulatory cycle. Poultry Sci. 80: (suppl. 1): 173.

Franzen, K. K., M. M. Beck, P. Y. Hester, G. Sarath, and N. Caceres, 2002. Calcium mobilization in the aging hen: I. ER-∝ populations in calcium-regulating tissues and skeletal integrity in three ages of laying hens. Poultry Sci. 81: (suppl. 1): accepted for presentation at the annual Poultry Science Association meeting in August 2002.

Schreiweis, M. A., J. I. Orban, M. C. Ledur, and P. Y. Hester, 2002. Assessment of densitometry in the measurement of bone mineral density and content of live White Leghorns fed varying levels of dietary calcium. Poultry Sci. 81: (suppl. 1): accepted for presentation at the annual Poultry Science Association meeting in August 2002.

Schreiweis, M. A., J. I. Orban, M. C. Ledur, and P. Y. Hester, 2002. The effect of the ovulatory cycle on bone mineral density and content in live White Leghorns as assessed by densitometry. Poultry Sci. 81: (suppl. 1): accepted for presentation at the annual Poultry Science Association meeting in August 2002.

Osteoporosis Project:
Completed schedule of events.

Date	Age of Birds	Event
Dec. 19-20, 2000	13 wk	Collect individual heparinized blood samples. Freeze whole blood
Dec. 21, 2000	13 wk	Individual body weights
Jan. 2-5, 2001	15 wk	Scan tibia and humerus of 35 birds (8 to 9 birds/day) Mark back feathers with black paint so that they can be housed close together in Room 2 of Layer
Jan. 18, 2001	17 wk	Scan tibia & humerus of 5 birds, bleed birds, euthanize birds, excise tibia & humerus
Jan. 19, 2001	17 wk	Scan excised bones from control diet

Mar. 5-9, 2001	24 wk	Scan tibia and humerus of 8 birds at 0, 5, 15, and 20 h following oviposition
Mar. 12-16, 2001	25 wk	Scan tibia and humerus of 35 birds (7 birds/day) Collect 2 eggs per bird prior to & following scan
March 29, 2001	27 wk	Scan tibia & humerus of 5 birds, bleed birds, euthanize birds, excise tibia & humerus, Collect 2 eggs per bird prior to scan
March 30, 2001	27 wk	Scan excised bones from control diet
April 16, 2001	30 wk	Scan tibia and humerus of 8 birds at 0, 5, 15, and 20 h following oviposition
May 4, 2001	32 wk	Begin hypocalcemic and hypercalcemic diets
May 21-25, 2001	35 wk	Scan tibia and humerus of 35 birds (7 birds/day) Collect 2 eggs per bird prior to & following scan
May 28-June 1, 2001	36 wk	Scan tibia and humerus of 30 birds (2 birds/day/diet or a total of 6 birds/day) Collect 2 eggs/bird prior to & following scan
June 7, 2001	37 wk	Scan tibia & humerus of 5 birds, bleed birds, euthanize birds, excise tibia & humerus, Collect 2 eggs per bird prior to scan
June 8, 2001	37 wk	Scan excised bones from control diet
June 13-14, 2001	38 wk	**Hypo- & hyper-calcemic diets**: Scan tibia & humerus of 5 birds/diet for a total of 15 birds, bleed birds, euthanize birds, excise tibia & humerus, Collect 2 eggs per bird prior to scan
June 15, 2001	38 wk	Scan excised bones from hypo- & hyper-calcemic diets
June 28, 2001	40 wk	Scan tibia and humerus of 8 birds at 0, 5, 15, and 20 h following oviposition

July 30- August 3, 2001	45 wk	Scan tibia and humerus of 30 birds (6 birds/day) Collect 2 eggs per bird prior to & following scan
August 6- 10, 2001	46 wk	Scan tibia and humerus of 30 birds (2 birds/day/diet or a total of 6 birds/day) Collect 2 eggs/bird prior to & following scan
August 16, 2001	47 wk	Scan tibia & humerus of 5 birds, bleed birds, euthanize birds, excise tibia & humerus, Collect 2 eggs per bird prior to scan
August 17, 2001	47 wk	Scan excised bones from control diet
August 22-23, 2001	48 wk	**Hypo- & hyper-calcemic diets**: Scan tibia & humerus of 5 birds/diet for a total of 15 birds, bleed birds, euthanize birds, excise tibia & humerus, Collect 2 eggs per bird prior to scan
August 24, 2001	48 wk	Scan excised bones from hypo- & hyper-calcemic diets
Oct. 8 – 12, 2001	55 wk	Scan tibia and humerus of 30 birds (6 birds/day) Collect 2 eggs per bird prior to & following scan
Oct. 15-19, 2001	56 wk	Scan tibia and humerus of 30 birds (2 birds/day/diet or a total of 6 birds/day) Collect 2 eggs/bird prior to & following scan
Oct. 25, 2001	57 wk	Scan tibia & humerus of 5 birds, bleed birds, euthanize birds, excise tibia & humerus, Collect 2 eggs per bird prior to scan
Oct. 26, 2001	57 wk	Scan excised bones from control diet
Oct. 31- Nov. 1, 2001	58 wk	**Hypo- & hyper-calcemic diets**: Scan tibia & humerus of 5 birds/diet for a total of 15 birds, bleed birds, euthanize birds, excise tibia & humerus, Collect 2 eggs per bird prior to scan
Nov. 2, 2001	58 wk	Scan excised bones from hypo- & hyper-calcemic diets

Dec. 17-21, 2001	65 wk	Scan tibia and humerus of 30 birds (6 birds/day) Collect 2 eggs per bird prior to & following scan
Jan. 3, 2002	67 wk	Scan tibia & humerus of 5 birds, bleed birds, euthanize birds, excise tibia & humerus, Collect 2 eggs per bird prior to scan
Jan. 4, 2002	67 wk	Scan excised bones from control diet
Jan. 17, 2002	69 wk	Induced molt
Feb. 25- Mar. 1, 2002	75 wk	Scan tibia and humerus of 30 birds (6 birds/day) Collect 2 eggs per bird prior to & following scan
March 14, 2002	77 wk	Scan tibia & humerus of 5 birds, bleed birds, euthanize birds, excise tibia & humerus, Collect 2 eggs per bird prior to scan
March 15, 2002	77 wk	Scan excised bones from control diet

Bone breaking strengths, bone ash, bone calcium and phosphorus have been determined. These data are currently being statistically analyzed.